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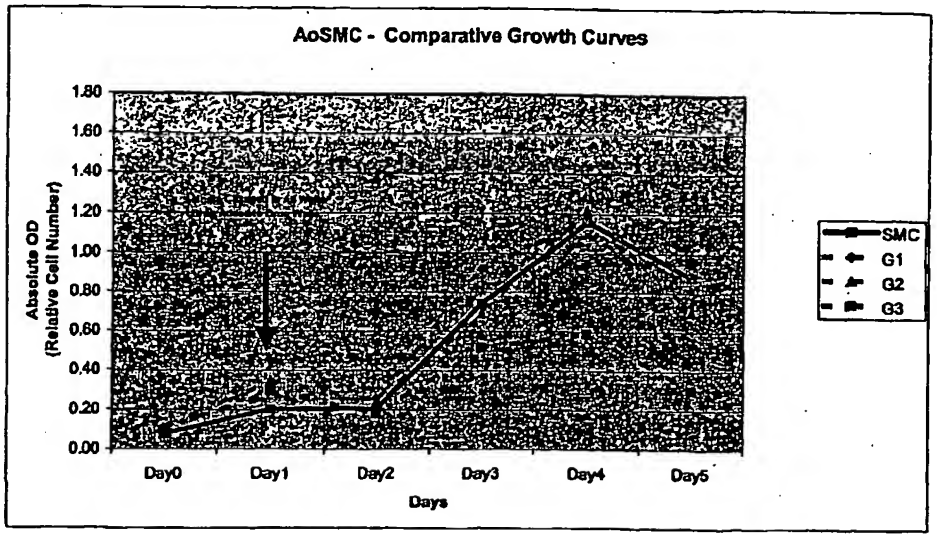
(54) Title: DRUG ELUTING VASCULAR STENT AND METHOD OF TREATING HYPERPROLIFERATIVE VASCULAR DISEASE

Imatinib Mesylate



(57) Abstract: This invention provides a drug eluting vascular stent and a method of preventing or treating hyperproliferative vascular disease in a mammal by administering an antiproliferative effective amount of imatinib mesylate, alone or in combination with other compounds, via a vascular stent.

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"DRUG ELUTING VASCULAR STENT AND METHOD OF TREATING HYPERPROLIFERATIVE VASCULAR DISEASE"

DESCRIPTION

FIELD OF THE INVENTION

This invention relates generally to vascular stents and, in particular, to a drug eluting vascular stent and a method of preventing or treating hyperproliferative vascular disease in a mammal by administering an antiproliferative effective amount of imatinib mesylate, alone or in combination with other compounds, via a drug eluting vascular stent.

BACKGROUND OF THE INVENTION

Many individuals suffer from heart disease, strokes, and loss of function or amputation of the lower extremities due to progressive atherosclerosis leading to severe blockage of the blood vessels that supply these organs with nutrients. Currently, a common treatment modality for these conditions is catheter-based mechanical opening of these blockages using balloon dilatation, plaque cutting (atherectomy), laser or other energy forms of treatment, metal prosthesis (stent) implantation or other mechanical means. In fact, over 80 percent of catheter-based coronary interventional procedures in US

utilize stent implantation. However, 20 to 40 percent of patients who undergo these procedures suffer from recurrence of the blockage (restenosis) within 3 to 6 month (Serruys PW et al. Circulation 1988; 77:361-371; stent restenosis references). In contrast to atherosclerosis, which is the most common biologic vascular disease that result from chronic arterial injury, the mechanical injury leading to restenosis is acute, and the course and pathologic findings of restenosis injury are considerably different from those of atherosclerosis. Restenosis injury occurs on a background of atherosclerosis, develops within 3 to 6 months following angioplasty, and features accumulation of smooth muscle cells and excessive extracellular matrix in the intima. Although localized intimal smooth muscle cell proliferation is present in lesions of human atherosclerosis, it is not the major component. In contrast, in the setting of postangioplasty restenosis, intimal proliferation of smooth muscle cells is the predominant feature (Clowes AW et al. Lab Invest 1983; 49:208-215; Austin GE, et al. J Am Coll Cardiol 1985; 6:359-375; other stent restenosis references).

The disease process is often referred to as a hyperproliferative vascular disease because of the patho-biology of this disease. Intimal thickening following

arterial injury can be divided into three sequential steps: 1) initiation of smooth muscle cell proliferation, 2) smooth muscle cell migration into the intimal, and 3) further proliferation of smooth muscle cells in the intima with deposition of extracellular matrix. Investigation of the pathogenesis of intimal thickening has shown that the arterial injury and the loss of endothelial cell integrity (Fishman JA, et al. Lan Invest 1977; 32:339-351) initiate a process in which platelets, macrophages and smooth muscle cells release paracrine and autocrine growth factors (such as platelet derived growth factor, epidermal growth factor, insulin-like growth factor, and transforming growth factor) and cytokines that result in the smooth muscle cell proliferation and migration (Deuel TF, et al. Annu Rev Cell Biol 1987; 3:433-492).

For over two decades, efforts have failed to identify effective pharmacological agents that can reduce restenosis (Faxon DP, et al. Cardiol Rev 1993; 1(4): 209-217). Recently, various ongoing experimental and clinical investigations have focused on local delivery of cytotoxic compounds that inhibit proliferation through arresting the cell cycle (rapamycin) or through interfering with the normal function of the cytosolic microtubular system leading to cell death (Paclitaxel). Both these agents seem to be effective in reducing smooth muscle cell

proliferation, however, they also suppress endothelial cell regeneration and function, an unfavorable effect that may lead to subsequent vascular thrombosis and spasm. Endothelial cell regeneration after arterial injury is particularly critical in cases of metal stent implantation in blood vessels where the continued exposure of metal surface to blood elements triggers vascular thrombosis that often leads to fatal events. In fact, the recent experience with intravascular radiation therapy for hyperproliferative vascular disease clearly illustrates the danger of delaying endothelial cell regeneration after stent implantation.

Therefore, a more favorable solution to this problem would be to identify a compound that inhibits smooth muscle cell proliferation without adversely affecting endothelial cell regeneration. Abundant evidence is available to support roles for growth factors as key signaling molecules in the accelerated vascular injury following angioplasty. Many features of the lesions can be readily explained by known properties of growth factors and other cytokines. Among the growth factors, it is remarkable that platelet derived growth factor (PDGF) alone has many of the properties needed to signal the pathologic features of lesions in restenotic arteries. For example, exposure of medial smooth muscle cells to PDGF

stimulates the transition of these cells from a quiescent to a proliferative phenotype. It is likely that PDGF is important in vivo, since it is released in significantly greater amounts from intimal smooth muscle cells isolated from arteries after balloon injury. The expression of PDGF occurs within sites and at times that are in accord with its potential to be a central regulator of accelerated vascular injury. Its properties as a growth factor and chemoattractant, together with its ability to transform cells, are the factors suspected to be responsible for the accelerated proliferation of smooth muscle cells and the local invasion of intimal tissues. Through its ability to upregulate expression of other cytokine genes, PDGF also extends the functions that it can direct to include other functions relevant to restenosis injury that cannot be understood when it is studied *in vitro*. For these reasons, PDGF is a leading candidate to play a major role in accelerated vascular injury. It is likely that many of the pathologic features of restenosis injury result from the continued high levels of expression of PDGF and related factors (Walker LN, et al. Atherosclerosis 1983; 47:123-130; Libby P, et al. N Engl J Med 1988; 318:1493-1498; Sjolund M, et al. J Cell Biol 1988; 106:403-413; Heldin CH et al. Physiological Reviews 1999; 79(4): 1283-1316).

PDGF exerts its effects on target cells by activating

two structurally related protein tyrosine kinase receptors (Matsui T, et al. Science 1989; 243; 800-803). The binding of PDGF to these receptors initiates potent mitogenic signals that result in cell growth, chemotaxis, actin reorganization, and prevention of apoptosis, all are critical to the formation of restenosis after vascular injury (Parsons JT, et al. Curr Opin Cell Biol 1997; 9:187-192; Kundra V, et al. Nature 1994; 367:474-476).

Imatinib mesylate is a protein-tyrosine kinase inhibitor that inhibits the Bcr-Abl tyrosine kinase, the constitutive abnormal tyrosine kinase created by the Philadelphia chromosome abnormality in chronic myeloid leukemia (CML). This compound has been approved by the Food and Drug Administration of the United States for use in patients with chronic myeloid leukemia.

However, *in vitro* studies demonstrate imatinib is not entirely selective; it also inhibits the receptor tyrosine kinases for platelet-derived growth factor (PDGF) and stem cell factor (SCF), c-Kit, and inhibits PDGF-and SCF-mediated cellular events (Buchdunger E, et al. JPET 2000; 295:139-145).

Imatinib mesylate, is designated chemically as 4-[(4-Methyl-1-piperazinyl)methyl]-N-[4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-phenyl]benzamide methanesulfonate. Imatinib mesylate is a white to off-

white to brownish or yellowish tinged crystalline powder. Its molecular formula is $C_{29}H_{31}N_7O \cdot CH_4SO_3$ and its relative molecular mass is 589.7. Imatinib mesylate is very soluble in water and soluble in aqueous buffers < pH 5.5 but is very slightly soluble to insoluble in neutral/alkaline aqueous buffers. In non-aqueous solvents, the drug substance is freely soluble to very slightly soluble in dimethyl sulfoxide, methanol and ethanol, but is insoluble in n-octanol, acetone and acetonitrile.

The following U.S. patents describe various drug eluting stents and methods of manufacture. These and all other patents referred to herein are hereby incorporated by reference in their entirety.

6,358,556 Drug release stent coating

6,258,121 Stent coating

6,206,915 Drug storing and metering stent

6,140,127 Method of coating an intravascular stent with an endothelial cell adhesive five amino acid peptide

6,120,847 Surface treatment method for stent coating

6,071,305 Directional drug delivery stent and method of use

5,980,551 Composition and method for making a biodegradable drug delivery stent

5,972,027 Porous stent drug delivery system

5,891,507 Process for coating a surface of a

metallic stent

5,891,108 Drug delivery stent

5,882,335 Retrievable drug delivery stent

5,837,313 Drug release stent coating process

5,769,883 Biodegradable drug delivery vascular stent

5,733,327 Stent for liberating drug

5,697,967 Drug eluting stent

5,599,352 Method of making a drug eluting stent

5,591,227 Drug eluting stent

5,551,954 Biodegradable drug delivery vascular stent

5,500,013 Biodegradable drug delivery vascular stent

5,464,450 Biodegradable drug delivery vascular stent

5,383,928 Stent sheath for local drug delivery

5,330,500 Self-expanding endovascular stent with silicone coating

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS 1A and 1B, FIGS 2A and 3B, and FIGS 3A and 3B are graphs illustrating the experimental results from *in vitro* experiments comparing the effects of Imatinib Mesylate, Rapamycin and Paclitaxel on human aortic smooth muscle cells (AoSMC) and human umbilical vein endothelial cells (HUVEC).

FIG 1A is a graph of comparative growth curves of untreated AoSMC cells and AoSMC cells treated with three

different concentrations of Imatinib Mesylate.

FIG 1B is a graph of comparative growth curves of untreated HUVEC cells and HUVEC cells treated with three different concentrations of Imatinib Mesylate.

FIG 2A is a graph of comparative growth curves of untreated AoSMC cells and AoSMC cells treated with three different concentrations of Rapamycin.

FIG 2B is a graph of comparative growth curves of untreated HUVEC cells and HUVEC cells treated with three different concentrations of Rapamycin.

FIG 3A is a graph of comparative growth curves of untreated AoSMC cells and AoSMC cells treated with three different concentrations of Paclitaxel.

FIG 3B is a graph of comparative growth curves of untreated HUVEC cells and HUVEC cells treated with three different concentrations of Paclitaxel.

DESCRIPTION OF THE INVENTION

This invention provides a method for preventing or treating hyperproliferative vascular disease in a mammal in need thereof by administering an antiproliferative effective amount of Imatinib Mesylate via a vascular stent impregnated with imatinib mesylate alone or in combination with other compounds. Alternatively or in addition, this drug may be delivered intravascularly, intranasaly,

intrabronchially, transdermally.

As such, imatinib mesylate is useful in treating intimal smooth muscle cell hyperplasia, restenosis, and vascular occlusion, particularly following either biologically or mechanically mediated vascular injury. Biologically mediated vascular injury includes, but is not limited to injury attributed to infectious disorders, atherosclerosis, and vascular injury resulting from hypothermia, and irradiation. Mechanical mediated vascular injury includes, but is not limited to vascular injury caused by percutaneous transluminal coronary angioplasty, atherectomy, laser, stent implantation, vascular surgery, transplantation surgery and other invasive procedures which disrupt the integrity of the vascular intima or the endothelium.

Treating includes retarding the progression, arresting the development, as well as palliation. Preventing includes inhibiting the development of and prophylacticly preventing of hyperproliferative vascular disease at the time of inducing vascular injury.

Imatinib mesylate was compared to rapamycin and paclitaxel in an *in vitro* standard pharmacological test procedure, which emulates the intimal smooth muscle cell proliferation observed following vascular injury. Also, we evaluated the effect of these drugs on endothelial cell

proliferation since the ability of endothelial cells to regenerate is a key feature to preserve the biologic integrity of the vessel wall. The experimental protocol and results of the experiment are described below.

The apparatus and methods of the present invention may be utilized with any of the numerous vascular stent designs and configurations known in the industry. Likewise, any of the various methods known in the industry for manufacturing and coating or impregnating stents with drugs or other biologically active substances, including those that have been incorporated by reference above, may be utilized for the manufacture of the drug impregnated stent of the present invention.

EXPERIMENTAL PROTOCOL

Contents:

I. Experimental Goals

1. Determine the optimal *in vitro* growth characteristics of human smooth muscle cell (SMC) and endothelial cell (EC) in culture at varying plating densities under standard culture conditions.

2. Compare the *in vitro* effects of varying drug concentrations of paclitaxel, rapamycin, and a novel prospective drug (imatinib mesylate) on growth characteristics for the same human SMCs and ECs under experimental conditions.

II. Experimental Design Overview

Experimental Goal (1)

•Human aortic smooth muscle cells (AoSMCs) and human umbilical vein endothelial cells (HUVECs) were acquired from professional sources and cultured x 2-3 population doublings. (refer to Section III below)

•These cells were independently plated on 96-well culture plates at varying cellular densities (2000 cells/cm^2, 5000 cells/cm^2, and 10,000 cells/cm^2) and prospectively cultured under standard conditions (refer to Section IV below)

•One plate for each given day and for each cell type (AoSMC and HUVEC) were analyzed for cellular proliferation

and viability using an MTS assay (Promega: CellTiter 96 AQueous-One Solution Assay) and 96-well automated optical density reader (refer to Section IX below)

•The daily growth characteristics for each cell type were analyzed using the above methods for a total of five days (refer to Section X below)

•Growth curves were plotted and compared in order to select the optimal conditions for subsequent studies

Experimental Goal (2)

•The three study drugs (rapamycin, paclitaxel, Drug X) were acquired from professional sources and dissolved in solvent to make stock solutions, which were then serially diluted in media to three study concentrations (10nM, 100nM, and 1000nM) (refer to Section V below)

•AoSMCs and HUVECs were plated independently under optimal conditions to a total of 24, 96-well culture plates (refer to Section VI below)

•The three drugs at three concentration each, solvent without drug at three concentrations, and standard media were added independently to cells on day 1 of the study only (refer to Sections VI and VII below)

•Cells in two columns (16 wells) for each cell type on each day will not receive drug and serve as internal controls (refer to Section VI below)

•Cellular viability and proliferation were assessed using the MTS assay for each cell type at each of the six time points (refer to Section IX below)

•Data were analyzed using the above methods to compare the growth altering effects of each of the three drugs at three concentrations, to each other and controls, for both AoSMCs and HUVECs (refer to Section X and XI below)

III Cell Line Acquisition and Pre-Experimental Culture

Cell Source

·Human aortic smooth muscle cells (AoSMCs) were purchased frozen from Clonetics/Biowhittaker/Cambrex (Item # CC-2571 / Lot # 0F0222)

·Company determined culture characteristics on arrival:

·Total Cell #: 917,500 / Viability: 95% / Doubling Time: 24-48 hrs

·Pooled human umbilical vein endothelial cells (HUVECs) were also purchased in frozen aliquot from Clonetics/Biowhittaker/Cambrex (Item # CC-2519 / Lot # 1F0832)

·Company determined culture characteristics on arrival:

·Total Cell #: 560,000 / Viability: 83% / Doubling Time: 18-48 hrs

Cell Culture

·Prior to experiments, both AoSMCs and HUVECS were thawed and independently propagated through 2-3 population doublings following Clonetics recommendations and standard cell culture technique

•Clonetics Growth Media and Reagents were used without alteration in all aspects of this study unless otherwise noted.

•SMGM contained: 500 ml SMBM-2 basal media, 5% FBS, and all recommended singlequot growth supplements (provided with SMGM-2 bulletkit)

•ECGM contained: 500 ml EBM basal media, 2% FBS, and all recommended singlequot growth supplements (provided with EGM-bulletkit)

IV Baseline Growth Characteristic Determination

General Goals:

•Determine the growth characteristics of AoSMCs and HUVECs cultured in 96-well plates at varying initial plating densities over 5 day time-period

•Select the optimal plating density for future experiments (i.e. that which exhibits exponential growth mid-time-period and reaches maximal confluency and overgrowth related inhibition by end-time-period

Note: Experiments detailed below were the same for both AoSMC and HUVEC

Cell Preparation

•Source cells were selected at 70-80 % confluency of

the second or third population doubling since initial thaw

•In order to synchronize cell cycle, source cells were changed from standard growth media to media containing 1% serum 24 hours prior to experiment (other growth factors were unchanged)

•On Day 0 of the experiment, source cells were removed from culture dishes by trypsinization (0.05 x 1-2 min), quantified by hemacytometer after centrifuge (800 RPM x 5 min), and re-suspended in media to obtain a stock solution of 25,000 cells/ml

96-well Cell Plating

•Cells from the stock solutions of both AoSMs and HUVEC were plated at three different densities (2000 cells/cm^2, 5000 cells/cm^2, and 10,000 cells/cm^2) using aseptic technique in the culture hood, and as detailed in the diagram below

•All wells except blanks were filled with appropriate media after cell delivery to equal 200 total microliters

•A separate 96-well plate was prepared for each Day 1-5

Experimental Culture

•After initial plating all 96-well plates were placed in a standard 37 degree, 5% CO_2 incubator

•Conditions, including media, were not changed except

as detailed below

Viability and Proliferation Assay

•For consecutive 24 hour time-periods, from Day 1 to Day 5, a single 96-well plate was removed from the incubator

•For this plate, culture media was removed from all wells and replaced with 100 microliters of MTS reagent / media solution (refer to Section IX below) and the plate was then placed back in the incubator

•3-4 hours later this plate was removed from the incubator and optical density data for each well was obtained using a 96-well ELISA reader (refer to Section IX below)

Data Analysis

•Absolute optical density was determined as (cellular OD - media only OD)

•Average absolute OD's for each plating concentration, for each day, and each cell type (AoSMC or HUVEC) were used to create temporal growth curves (refer to Section X and XI below)

•From these growth curves (data not shown) it was determined that a plating density of 10,000 cells/cm2 was optimal for both AoSMCs and HUVECs studied in 96-well plates over a 5 day time-period

V **Drug Acquisition and Solution Preparation**

Drug Sources

•Rapamycin was purchased from Sigma Aldrich as a 1 mg powder (Item # R0395)

•Paclitaxel was purchased from Sigma Aldrich as a 5 mg powder (Item # T7191)

•Imatinib mesylate was purchased from the Stanford University Pharmacy under *in vitro* experimental protocol

Stock Solution

•All three drugs have been shown to be soluble in DMSO which was selected as the universal solvent

•Under aseptic conditions and in their original containers, rapamycin and paclitaxel were dissolved in the appropriate amount of standard DMSO/media freezing solution to yield a 10 mM stock solution

•Under similar conditions, Imatinib mesylate was less soluble in the DMSO/media solution, requiring a stock concentration of 1 mM for full solubility

Serial Dilutions

•The stock solutions for each of the three drugs were serially diluted in both AoSMC growth media and HUVEC growth media to obtain the three experimental drug

concentrations (10 nM, 100 nM, and 1000 nM = 1μM)

VI Drug Study Cell Plating

Cell Preparation

·Source cells were selected at 70-80 % confluency of the third or fourth population doubling since initial thaw

·In order to synchronize cell cycle, source cells were changed from standard growth media to media containing 1% serum 24 hours prior to experiment (other growth factors were unchanged) and media containing 1% serum was used for the remainder of the experiment

·On Day 0 of the experiment, source cells were removed from culture dishes by trypsinization (0.05 x 1-2 min), quantified by hemacytometer after centrifuge (1000 RPM x 5 min), and resuspended in media to obtain a stock solution of 25,000 cells/ml

96-well Cell Plating

·Cells from the stock solutions of both AoSMs and HUVEC were plated at a density of 10,000 cells/cm^2 using aseptic technique in the culture hood, and as detailed in the diagram following (refer to Section VII below)

·On Day 0, all wells except blanks were filled with appropriate media after cell delivery to equal 200 total microliters

•Two 96-well plates were prepared for each cell type (AoSMC and HUVEC) and for each Day 0-5 for a total of 24 plates

Note: All experiments were the same for both AoSMCs and HUVECs

VII Drug Administration

Drug Delivery

•On Day 1, 24 hrs after initial plating, media was removed from all wells by careful vacuum suction technique

•This was replaced with the appropriate growth media-drug solution for each of three drugs at three concentrations (detailed previously) as diagramed below

•For each cell type (AoSMC or HUVEC), and each day, two 96-well plates were required to incorporate all three drugs (24 total plates)

•In order to assess potential independent effect of the solvent (DMSO) on proliferation, the three solvent concentration (in appropriate media and corresponding to the solvent component of the three drug concentrations) were added for each day as diagrammed below

•Standard wells contained only media and were used for OD control in each individual plate during analysis

•Control wells contained the plate specific cells

without drug or solvent, and served as the control for all drug effects on a given day for a given cell type

•All wells were appropriately filled to a total of 200 microliters

Note: All experiments were the same for both AoSMCs and HUVECs

VIII Experimental Culture Maintenance

Experimental Culture

•After initial plating all 96-well plates were placed in a standard 37 degree, 5% CO_2 incubator

•Media was changed on Day 1 only, as detailed above

•Drug was added on Day 1 only, as detailed above

•Individual plates were kept in the incubator from Day 1 until the time of their analysis, and aseptic conditions were maintained

•Plates were reviewed under inverted light microscope immediately prior to MTS assay for visual confirmation of growth characteristics

•Gross overgrowth was apparent by Day 5 in all plates for both AoSMC and HUVEC cell types

IX Viability and Proliferation Assay

•For consecutive 24 hour time-periods, from Day 0 to

Day 5, four 96-well plates were removed from the incubator (2 plates each for AoSMCs and HUVECs)

•For these plates, culture media was removed from all wells and replaced with 100 microliters of MTS reagent solution

•This solution contained 20 microliters of Promega: CellTiter 96 Aqueous One reagent, in 80 microliters of cell-appropriate growth media for each well

•Promega recommendations for assay use were followed throughout, including reagent administration under dark lighting

•After addition of reagent, plates were replaced in the incubator

•3-4 hours later these plates were removed from the incubator and optical density data for each well or each plate was obtained using a 96-well automated ELISA reader

•Plates were read within 1.5 hrs of the same time each day

X Analysis of Data

Optical Density Data

- Data was converted to a Microsoft Excel spreadsheet for all further analysis
- A sample of optical density data for AoSMC on Day 3 is presented below:

- The degree of variability within a given column in this example is consistent with data from all plates (statistical analysis not presented)
- Rarely, OD data for a well was significantly different from other values in a given column to be considered an error or outlier, and these data points were removed prior to computational analysis (empty spaces above)

Computational Analysis

- Optical Densities for each column were averaged (n=8 with rare exceptions for data outliers as demonstrated previously)
- With the MTS assay steps detailed above, the 'standard' wells contained only MTS reagent in media at the time of analysis
- Average OD values for these 'standard' columns were then subtracted from those column averages of drug treated cells, in order to obtain an absolute OD for drug treated cells
- (drug-cell + media + reagent) - (media + reagent) = ((drug-cell)abs)
- Average OD values for these 'standard' columns were also subtracted from those columns containing control cells in order to obtain an absolute OD for control cells

•(cont-cell + media + reagent) - (media + reagent) = ((cont-cell)abs)

•Average absolute OD's for cells at a given drug concentration, were then plotted for Days 0-5 for both AoSMCs and HUVECs using Microsoft Excel

•Average absolute OD's for AoSMC and HUVEC control cells were plotted on the same respective graphs for Days 0-5

EXPERIMENTAL RESULTS

FIGS 1A and 1B, FIGS 2A and 3B, and FIGS 3A and 3B are graphs illustrating the experimental results from *in vitro* experiments according to the experimental protocol described above comparing the effects of Imatinib Mesylate, Rapamycin and Paclitaxel on human aortic smooth muscle cells (AoSMC) and human umbilical vein endothelial cells (HUVEC).

FIG 1A is a graph of comparative growth curves of untreated AoSMC cells and AoSMC cells treated with three different concentrations of Imatinib Mesylate. The graph shows inhibition of smooth muscle cell proliferation by Imatinib Mesylate at a concentration of 1000 nM.

FIG 1B is a graph of comparative growth curves of untreated HUVEC cells and HUVEC cells treated with three different concentrations of Imatinib Mesylate. The graph

shows no inhibition of endothelial cell growth at all concentrations of Imatinib Mesylate.

FIG 2A is a graph of comparative growth curves of untreated AoSMC cells and AoSMC cells treated with three different concentrations of Rapamycin. The graph shows inhibition of smooth muscle cell proliferation at all concentrations of Rapamycin.

FIG 2B is a graph of comparative growth curves of untreated HUVEC cells and HUVEC cells treated with three different concentrations of Rapamycin. The graph shows inhibition of endothelial cell growth at all concentrations of Rapamycin.

FIG 3A is a graph of comparative growth curves of untreated AoSMC cells and AoSMC cells treated with three different concentrations of Paclitaxel. The graph shows inhibition of smooth muscle cell proliferation at all concentrations of Paclitaxel.

FIG 3B is a graph of comparative growth curves of untreated HUVEC cells and HUVEC cells treated with three different concentrations of Paclitaxel. The graph shows inhibition of endothelial cell growth at all concentrations of Paclitaxel.

In summary, this *in vitro* study illustrates that imatinib mesylate inhibits smooth muscle cell proliferation at a concentration of 1000 nM without

affecting endothelial cell viability. On the other hand, both rapamycin and paclitaxel inhibit both smooth muscle cells and endothelial cells even at lower concentrations. This differential effect of imatinib mesylate makes it a unique drug that may decrease intimal proliferation without affecting endothelial healing. This effect makes it preferable to the other agents under investigation.

When imatinib mesylate is employed alone or in combination with other compounds in the prevention or treatment of hyperproliferative vascular disease, it can be formulated neat or with a pharmaceutical carrier to a mammal in need thereof. The pharmaceutical carrier may be solid or liquid.

A solid carrier can include one or more substances which may also act as flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, methyl cellulose, sodium carboxymethyl cellulose, polyvinylpyrrolidine, low melting waxes and ion exchange resins.

Liquid carriers are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active ingredient can be dissolved or

suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid carrier can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers or osmo-regulators.

Imatinib mesylate, alone or in combination with other compounds can be administered intravascularly or via a vascular stent impregnated with imatinib mesylate, alone or in combination with other compounds, during balloon dilatation and stent implantation to provide localized effects immediately following injury.

CLAIMS

1. Use of imatinib mesylate, alone or in combination with other compounds, for the preparation of a vascular stent for the treatment of a hyperproliferative vascular disease.

2. Use according to claim 1, wherein the said hyperproliferative vascular disease is caused by vascular injury, percutaneous transluminal coronary angioplasty, laser or other energy form treatment, atherectomy treatment, stent implantation, vascular surgery.

3. Use according to claim 1 or to claim 2, wherein the said vascular stent comprises an antiproliferative effective amount of imatinib mesylate.

4. Use according to any one of claims 1 to 3, wherein the said vascular stent is impregnated with the said imatinib mesylate.

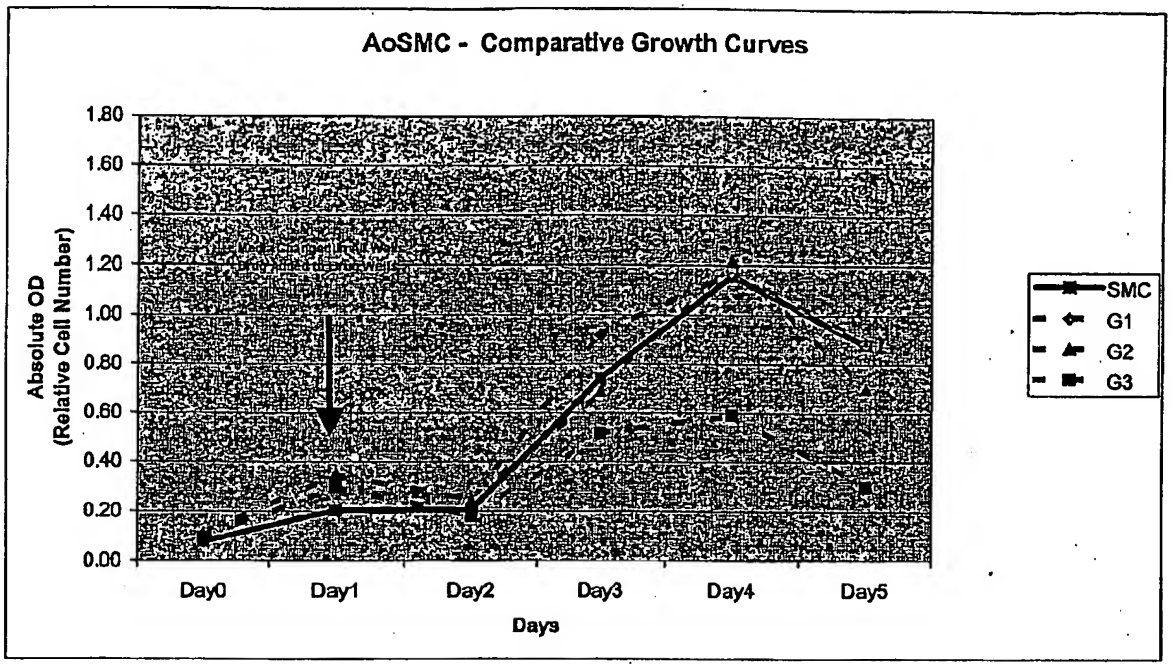
5. Apparatus for treating a hyperproliferative vascular disease, comprising a vascular stent which comprises imatinib mesylate alone or in combination with other compounds.

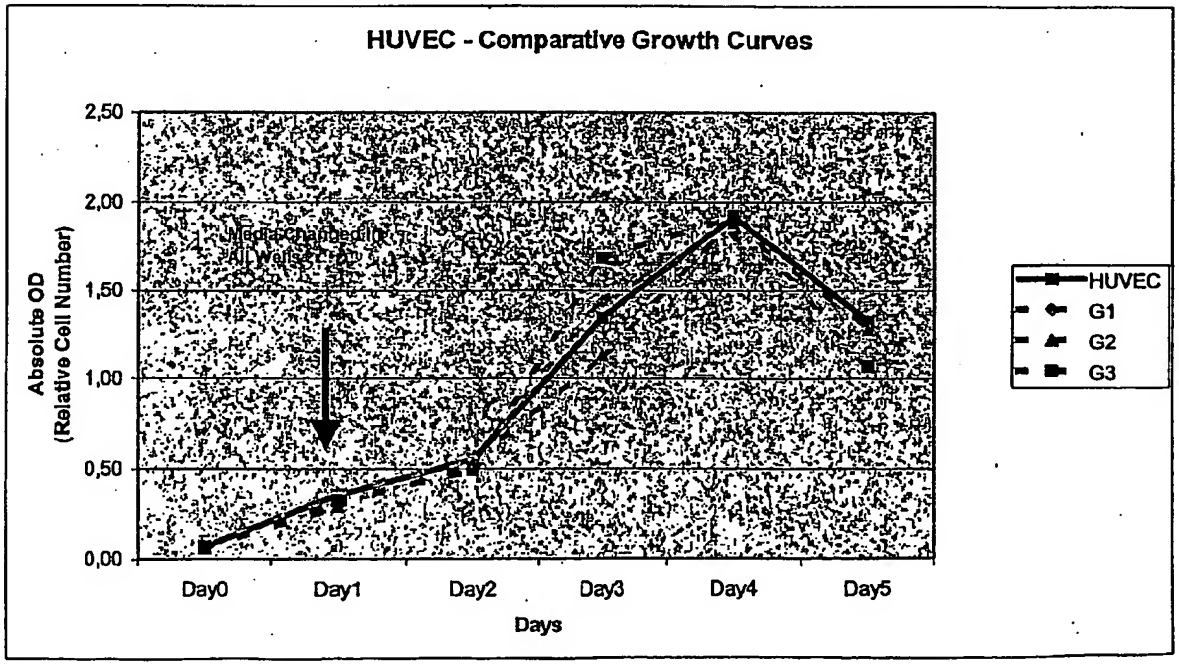
6. Apparatus according to claim 5, wherein the said hyperproliferative vascular disease is caused by vascular injury, percutaneous transluminal coronary angioplasty, laser or other energy form treatment, atherectomy treatment, stent implantation, vascular surgery.

7. Apparatus according to claim 5 or to claim 6, wherein the said vascular stent is as defined in claim 3 or in claim 4.

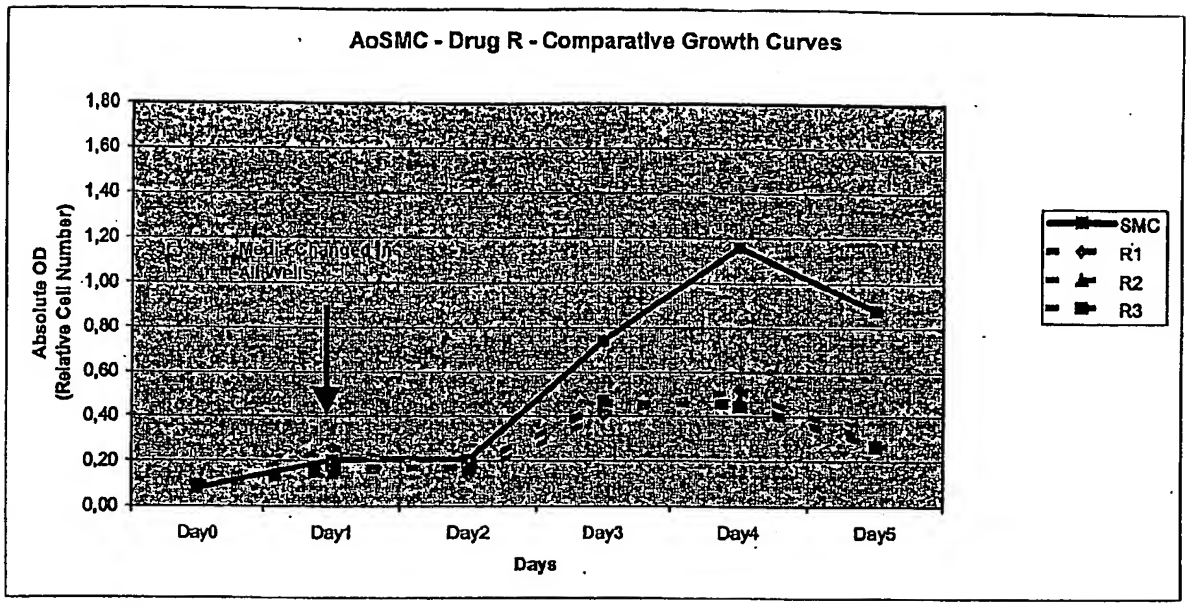
8. Use of imatinib mesylate, alone or in combination with other compounds, for the preparation of a pharmaceutical composition to be applied on a vascular stent.

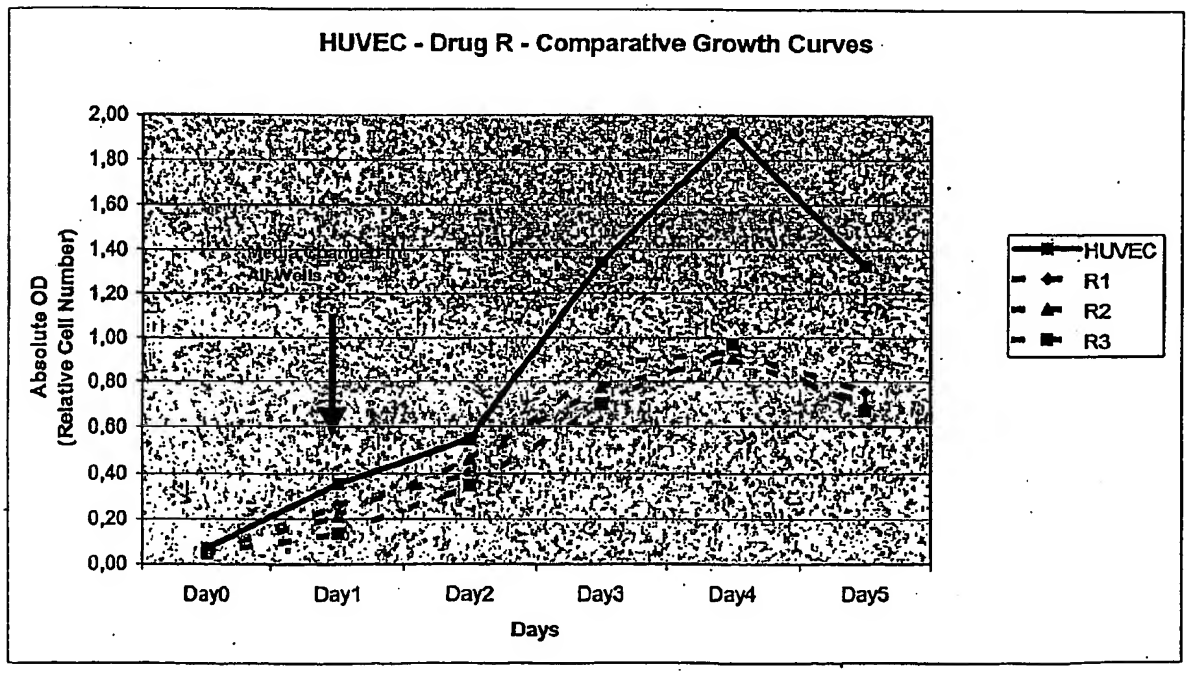
FIGS 1A and 1B
Imatinib Mesylate



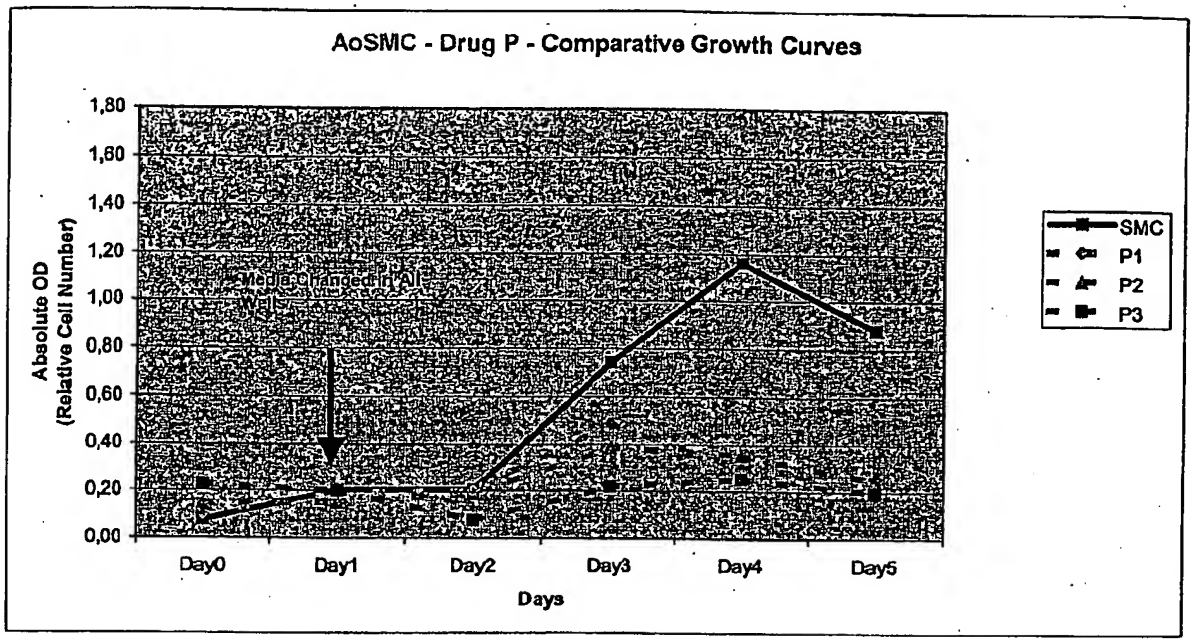


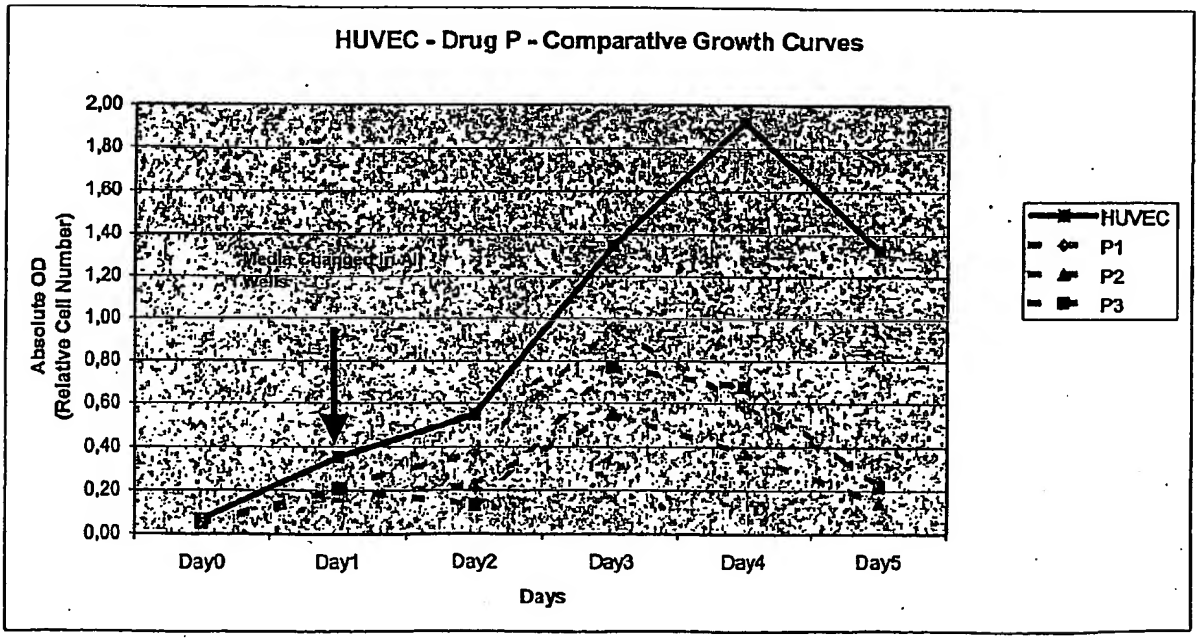
FIGS 2A and 2B
Rapamycin





FIGS 3A and 3B
Paclitaxel





INTERNATIONAL SEARCH REPORT

PCT/IB 03/01230

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61L31/16 A61K31/506

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61L A61K C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, FSTA, INSPEC, COMPENDEX, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 99 03854 A (NOVARTIS ERFIND VERWALT GMBH ;NOVARTIS AG (CH); BUERGER HANS MICHA) 28 January 1999 (1999-01-28) page 1, paragraph 1 page 9, paragraph 3 page 14, paragraph 2 page 17, paragraph 2	1-8
Y	US 2001/032014 A1 (STANSLASKI JOEL L ET AL) 18 October 2001 (2001-10-18) page 1, right-hand column, paragraph 10 page 3, left-hand column, paragraph 29	1-8

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 July 2003	30/07/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Menidjel, R

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